Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US04/043383

International filing date: 23 December 2004 (23.12.2004)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/533,467
Filing date: 29 December 2003 (29.12.2003)

Date of receipt at the International Bureau: 03 February 2005 (03.02.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)

World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1276699

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

January 22, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: *60/533,467*
FILING DATE: *December 29, 2003*
RELATED PCT APPLICATION NUMBER: *PCT/US04/43383*

Certified by

Jon W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

PTO/SB/16 (05-03)
Approved for use through 4/30/2003. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label N . EV 405 279 804 US

INVENTOR(S)

Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Allan	**Mishra**	**Menlo Park, California**

☐ *Additional inventors are being named on the separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

PATIENT INDIVIDUALIZED TRANSDERMAL PATCH

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☒ Customer Number 24353 →

OR *Type Customer Number here*

Place Customer Number Bar Code Label here

☐ Firm *or* Individual Name	**Bozicevic, Field & Francis LLP**				
Address	**200 Middlefield Road, Suite 200**				
Address					
City	**Menlo Park**	State	**California**	Zip	**94025**
Country	**U.S.A.**	Telephone	**(650) 327-3400**	Fax	**(650) 327-3231**

ENCLOSED APPLICATION PARTS (*check all that apply*)

☒ Specification *Number of Pages* 33 ☐ CD(s), Number

☒ Drawing(s) *Number of Sheets* 5 ☒ Other (specify) postcard

☒ Application Data Sheet. See 37 CFR 1.76

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT

☒ Applicant claims small entity status. See 37 CFR 1.27.

☐ A check or money order is enclosed to cover the filing fees

☒ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 50-0815

☐ Payment by credit card. Form PTO-2038 is attached.

FILING FEE AMOUNT$ 80.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☒ No

☐ Yes, the name of the U.S. Government agency and the Government contract number are: _____

Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME Karl Bozicevic

Date 12/29/2003

REGISTRATION NO. 28,807 *(if appropriate)*

Docket Number: AMBI-006PRV

TELEPHONE (650) 327-3400

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Atty. Docket: AMBI-006PRV
December 29, 2003

PATIENT INDIVIDUALZED TRANSDERMAL PATCH

FIELD OF THE INVENTION

[0001] This invention relates generally to the field of transdermal drug delivery and specifically to a transdermal device loaded with an active compound for the patient being treated.

BACKGROUND OF THE INVENTION

[0002] The transdermal route of parenteral delivery of drugs provides many advantages and transdermal systems for delivering a wide variety of drugs or other beneficial agents are described in U.S. Pat. Nos. 3,598,122, 3,598,123, 4,286,592, 4,314,577, 4,379,454 and 4,568,343 for example, all of which are incorporated herein by reference.

[0003] In these devices, a drug or other active agent is released by diffusion from a reservoir through the agent releasing surface of the device to the biological environment at which the device is applied. Such devices perform well in the administration of many agents but are generally not loaded with drugs or active compounds specific to the patient being treated.

[0004] Non-diffusional devices are known which do not immediately present drug to the biological environment when installed, such as devices which contain material in breakable microcapsules, or fluid imbibing pumps, such as that described in U.S. Pat. No. 4,655,766 of Theeuwes et al.

[0005] The devices of this invention are particularly useful in providing a predetermined delayed onset of therapeutic effect for any desired time period after application to the skin. Thus a device could be removed and a new one applied simultaneously, wherein the desired drug-free interval is obtained.

[0006] One of the advantages of a continuous release dosage form, such as a transdermal drug delivery device, is the improvement in patient compliance that is obtained from the concurrent removal of one device and application of a new device at the same time. This advantage is lost when removal and application occur at different times or where onset of a therapeutic effect is desired at an inconvenient time such as shortly prior to arousal from sleep. It is not possible, using concurrent application and removal of diffusional delivery devices known in the art, to

substantially delay the onset of transdermal drug delivery from the time of application, such as bedtime, until shortly prior to arousal.

[0007] A common problem encountered with state of the art systems is how to deal with unstable active agents, especially those that tend to degrade the adhesive and other system components. Therefore, there is a continuing need for a transdermal therapeutic system that provides stability of the adhesive and all components during storage.

[0008] The skin is particularly useful as it presents large areas for drug administration, as the skin is the largest organ of the body. The utility of such a mode of administration has been promoted with the discovery and development of a group of compounds that promote transdermal penetration of the various active drugs. Such compounds are known in the art as penetration enhancers. They are generally characterized to be from the group of monovalent branched or unbranched aliphatic, cycloaliphatic or aromatic alcohols of 4-12 carbon atoms; cycloaliphatic or aromatic aldehydes or ketones of 4-10 carbon atoms, cycloalkanoyl amides of C 10-20 carbons, aliphatic, cycloaliphatic and aromatic esters, N, N-di-lower alkylsulfoxides, unsaturated oils, terpenes and glycol silicates.

[0009] These compounds and their specific activity as penetration enhancers, are more fully discussed in the text "Transdermal Delivery of Drugs, A. F. Kydonie US (Dd)-1987 CRL Press and in such as U.S. Pat. Nos. 4,913,905, 4,917,676 and 5,032,403.

[0010] As a result of these penetration enhancers, almost any drug, to some degree, can be administrated transdermally cf. U.S. Pat. Nos. 4,917,676, 3,598,122; 3,598,123; 3,742,951; 3,797,494; 3,948,254; 3,996,734; 4,284,444; and 4,597,961. Examples of such pharmacological actives include administration of antibacterial such as the penicillins, tetracyclines, second and third generation cephalosporins, chlor-amphenicol sulfonamides; sedatives and/or hypnotics, such as barbiturates, carbromal, antijussives such as codeine and dextromethorphan; anti-anxiety drugs such as the benzodiazepines including diazepam, buspirone; psychostimulants such as imipramine amitriptyline and other tricyclic anti-depressants; anti psychotic drugs and tranquilizers such as lithium, chlorpromazine and haloperidol, reserpine, thiopro-pazate; Parkinsonism control agents such as bromotriptine, percolide, the anticholmergics including benzotropine, pro-cyclidine, amantadine (also an

antiviral); hormones and hormone antagonists and agonists, including adrenocortico-steroids; insulin, androgenic steroids, estrogenic and pro-gestrogenic steroids, thyroxin and its agonist 5-FU(fluoro-uracil), tamoxifen; antipvretics and analgesics such as aspirin/acetaminophen and other non-steroidal anti-inflammatory drugs (NSAID), analgesics based on morphine; morphine antagonists; vasodilating agents such as nitro-glycerine, isorbide dinitrate; alpha beta-blockers and other cardioactive drugs; antimalarials; anti-histamines and anti-cholinergics including atropine hyoscyamine or methscopalo-mine (for motion sickness; weaning agents such as nicotine for addiction to tobacco; and antiasthmatic bronchodilators such as formoterol; and combinations of such pharmaceutical actives.

[0011] Of course, while feasible, not all of these actives have yet been completely tested for efficacy by transdermal administration but many are under vigorous scrutiny. Other actives at this time are not economically viable for such administration, as the cost of full safety testing is too great for the specific number of patients involved.

[0012] As can be seen from this background discussion and the history of this type of medication, it is apparent that application by transdermal patch is a useful form for the administration of medication.

[0013] The evolution of transdermal drug delivery has centered around patch technology. Patch technology is based on the ability to hold an active ingredient in constant contact with the epidermis over substantial periods of time, certain drug molecules, held in such a state, will eventually transfer from the patch into the skin and to a small and variable degree, into the bloodstream. Thus, patch technology relies on the ability of the human body to passively pick up drug molecules through the skin. Transdermal drug delivery using patch technology has recently been applied for delivery of nicotine, in an effort to assist smokers in quitting, the delivery of nitroglycerine to angina sufferers, the delivery of replacement hormones (e.g., estrogen, progesterone and testosterone) and for the delivery of scopolamine for motion sickness. These drug delivery systems comprise a patch with an active ingredient such as a drug incorporated therein, the patch also includes an adhesive for attachment to the skin so as to place the active ingredient in close proximity to the skin.

[0014] In an effort to enhance the efficiency of transdermal drug delivery, the prior art teaches that by mixing certain individual ingredients (penetration enhancers) with a drug molecule, the ability of the drug molecule to pass through the skin is increased somewhat. For example, U.S. Pat. No. 4,933,184 discloses the use of menthol as a penetration enhancer; U.S. Pat. No. 5,229,130 discloses the use of vegetable oil (soybean and/or coconut oil) as a penetration enhancer; and U.S. Pat. No. 4,440,777 discloses the use of eucalyptol as a penetration enhancer.

SUMMARY OF THE INVENTION

[0015] A transdermal patch is disclosed and described as is a method of treatment using the patch. The patch can be produced in a range of different shapes and configurations including a face mask shape which fits onto the user's face or a portion thereof. The patch may be produced in an unloaded or drug empty form and loaded with the drug or active ingredient specific for the patient being treated. The active drug may be an isolated component of the patient's blood which has been treated and formulated. For example, platelets are concentrated and sonicated to create a platelet releasate which is formulated to adjust the pH and added to the unloaded patch which is placed on the patient's skin. The patient treated may be the same patient from which the blood component such as the platelets were obtained.

[0016] An aspect of the invention is an unloaded transdermal patch comprising a self-sealing portion and/or area which is opened and then resealed after a drug is added.

[0017] Another aspect of the invention is a transdermal patch loaded with an active ingredient in the form of a component of blood obtained from the same patient to be treated with the patch.

[0018] Still another aspect of the invention is a transdermal patch have a two dimensional shape and a three dimensional configuration design to fit to a patient's face or a potion of the patient's face.

[0019] Yet another aspect of the invention is a method of doing business whereby platelets extracted from a patient are sonicated and the releasate formulated and put into a transdermal patch which is provided back to the patient for treating the patient's skin and reducing wrinkles and/or their appearance.

[0020] These and other objects, advantages, and features of the invention will become apparent to those persons skilled in the art upon reading the details of the carious embodiments and aspects of the invention as more fully described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] The invention is best understood from the following detailed description when read in conjunction with the accompanying drawings. It is emphasized that, according to common practice, the various features of the drawings are not to-scale. On the contrary, the dimensions of the various features are arbitrarily expanded or reduced for clarity. Included in the drawings are the following figures:

[0022] Figure 1 is a graph of cell count versus time for cultured fibroblast cells in PRP.

[0023] Figure 2 is a graph of cell count for three different concentrations of PRP releasate and a control.

[0024] Figure 3 is a graph of cell counts over seven days for a control and a culture with sonocated PRP.

[0025] Figure 4 is a first type of transdermal patch, according to the invention, with a permeable membrane.

[0026] Figure 5 is a second type of transdermal patch, according to the invention, without a permeable membrane.

[0027] Figure 6 is a plain schematic view of a face mask embodiment of a transdermal patch of the invention

[0028] Figure 7 is a schematic cross-sectional view of a portion of the patch of Figure 6.

DETAILED DESCRIPTION OF THE INVENTION

[0029] Before the present transdermal patch, formulations and methods are described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

[0030] Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limits of that range is also specifically disclosed. Each smaller range between any stated value or intervening value in a stated range and any other stated or intervening value in that stated range is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included or excluded in the range, and each range where either, neither or both limits are included in the smaller ranges is also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

[0031] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

[0032] It must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a patch" includes a plurality of such patches and reference to "the formulation or method" includes reference to one or more formulations, methods and equivalents thereof known to those skilled in the art, and so forth.

[0033] The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DEFINITIONS

[0034] The term "platelet" is used here to refer to a blood platelet. A platelet can be described as a minisule protoplasmic disk occurring in vertebrate blood. Platelets play a role in blood clotting. The platelet may be derived from any source including a human blood supply, or the patient's own blood. Thus, the platelets in the composition of the inventions may be autologous. The platelets may be homologous, i.e. form a human but not the same human being treated with the composition.

[0035] The term "platelet-rich-plasma," "PRP" and the like are used interchangeable here to mean a concentration of platelets in a carrier which concentration is above that of platelets normally found in blood. For example, the platelet concentration may be 5 times, 10 times, 100 times or more the normal concentration in blood. The PRP may use the patient's own plasma as the carrier and the platelets may be present in the plasma at a range of from about 200,000 or less to 2,000,000 or more platelets per cubic centimeter. The PRP may be formed from whole blood e.g. by technology disclosed in any of 5,614,106; 5,580,465; 5,258,126 or publication cited in these patents and if needed stored by technology as taught in 2002/0034722A1; 5,622,867 or publications cited therein. The PRP may comprise blood component other than platelets. It may be 50% or more, 75% or more, 80% or more, 95% or more, 99% or more platelets. The non-platelet components may be plasma, white blood cells and/or any blood component. PRP is formed from the concentration of platelets from whole blood, and may be obtained using autologous, allogenic, or pooled sources of platelets and/or plasma. PRP may be formed from a variety of animal sources, including human sources.

[0036] The "dose" of platelets administered to a patient will vary over a wide range based on the age, weight, sex and condition of the patient as well as the patients' own normal platelet concentration, which as indicated above can vary over a ten fold or greater range. Doses of 1 million to 5 million platelets are typical but may be less or greater than such by a factor of two, five, ten or more.

[0037] The term "platelet releasate" is the PRP as defined above but treated so that what is inside the platelet shells is allowed to come out. The releasate may be subjected to processing whereby the platelet shells are removed and/or other blood components are removed, e.g. white blood cells and/or red blood cells or remaining

plasma is removed. The pH of the platelet releasate may be adjusted to physiological pH or higher or to about 7.4 ± 10%, 7.4 ± 5%, 7.4 ± 2% or 7.4 to 7.6 as needed.

[0038] The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacologic, physiologic or cosmetic effect. The effect may be prophylactic in terms of completely or partially preventing a condition, appearance, disease or symptom thereof and/or may be therapeutic in terms of a partial or complete cure for a condition and/or adverse effect attributable to a condition or disease. "Treatment" as used herein covers any treatment of a condition, disease or undesirable appearance in a mammal, particularly a human, and includes:

(a) preventing the disease (e.g. cancer), condition (pain) or appearance (e.g. visable tumors) from occurring in a subject which may be predisposed to such but has not yet been observed or diagnosed as having it;

(b) inhibiting the disease, condition or appearance, i.e., causing regression of condition or appearance.

(c) relieving the disease, condition or undesired appearance, i.e., causing regression of condition or appearance.

[0039] The invention includes treating patients with platelet releasate or components thereof formulated in accordance with the invention. Accordingly, the term "treatment' is intended to mean providing a therapeutically detectable and beneficial effect of any kind on a patient.

[0040] The terms "synergistic", "synergistic effect" and like are used herein to describe improved treatment effects obtained by combining one or more active components together in a composition or in a method of treatment. Although a synergistic effect in some field is meant an effect which is more than additive (e.g., 1+1=3) in the field of treating many diseases an additive (1+1=2) or less than additive (1+1=1.6) effect may be synergistic. For example, if one active ingredient removed 50% of a disease and a second active ingredient removed 50% of the disease the combined (and merely additional) effect would be 100% removal of the disease. However, the effect of both would not be expected to remove 100% of the disease. Often, two active ingredients have no better or even worse results than

either component by itself. If an additive effect could be obtained merely by combining treatments than multiple ingredients could be applied to successfully treat any disease and such is not the case.

[0041] The term "iontophoresis" means the migration of ionizable molecules through a medium driven by an applied low level electrical potential. This electrically mediated movement of molecules into tissues and in particular into the skin is in addition to the movement obtained via concentration gradient dependent diffusion. If the tissue (e.g. skin) through which the molecules travel also carries a charge, some electro-osmotic flow occurs. However, generally, the rate of migration of molecules with a net negative charge towards the positive electrode and vice versa is determined by the net charge on the moving molecules and the applied electrical potential. The driving force may also be considered as electrostatic repulsion. Iontophoresis usually requires relatively low constant DC current in the range of from about 2-5 mA. For enhancing the delivery of a formulation of the invention such and a platelet releasate through the skin (transdermal iontophoresis), one electrode is positioned over the treatment area and the second electrode is located at a remote site, usually somewhere else on the skin. The return electrode may, for certain applications, be placed elsewhere on the skin as the iontophoretic delivery electrode. With the present invention the return electrode may be similarly positioned on the skin. The applied potential for iontophoresis will depend upon number of factors, such as the electrode configuration and position on the tissue (skin), the nature and charge characteristics of the molecules (e.g. releasate formulation) to be delivered, and the presence of other ionic species within components of the patch and in the tissue extracellular compartments.

[0042] As used herein "Collagen" means pharmaceutical grade collagen used in the treatment of human patients. Collagen is a fibrous protein that form fibrils having a very high tensile strength and that has been found in most multicellular organisms. Collagen serves to hold cells and tissues together and to direct the development of mature tissue. Collagen is the major fibrous protein in skin, cartilage, bone, tendon, blood vessels and teeth.

[0043] There are many types of collagen which differ from each other to meet the requirements of various tissues. Some examples of types of collagen are as follows:

type one $[\alpha 1(I)]_2 \alpha 2$ which is found in skin, tendon, bone and cornea; type two $[\alpha 1(II)_3$ which is found in cartilage intervertebral disc, and the vitreous body; type three $[\alpha 1(III)]_3$ which can be found in skin and the cardiovascular system; type four $[\alpha 1(IV)]_2$ $\alpha 2(IV)$ which can be found in basement membrane; type five $[\alpha 1(V)]_2$ $\alpha 2(V)$ and $\alpha 1(V)\alpha 2(V)\alpha 3(V)$ which is found in the placenta and cornea. Examples of newly identified forms of collagen include: type seven (VII) which is found in anchoring fibrils beneath many epithelial; and types nine (IX), ten (X) and eleven (XI), which are minor constituents of cartilage.

[0044] The chemical characterization of native collagen was difficult since its low solubility made isolation of collagen a tedious task. Eventually, it was discovered that collagen from tissues of young animals was not as extensively cross linked as that of mature tissues and thus was more amenable to extraction. For example, the basic structural unit of type I collagen, tropo-collagen, can be extracted in intact form from some young, collagen-containing animal tissues.

[0045] Substantial information can be found in patents and publications relating to uses of Collagen. For example, see US patents 4,294,241; 4,668,516; 5,640,941; and 5,716,411 all of which are incorporated herein by reference as are the publications and patents cited in these patents to disclose and describe various ways of using collagen which can in turn be mixed with and administered and used with platelet formulations of the present invention.

ADDITIONAL ACTIVE COMPONENTS

[0046] There are a number of compounds which can have a beneficial effect on treating skin. The effect of those components can be enhanced when combined in a composition of the invention. For example, further beneficial results may be obtained by combining the compositions according to the invention with at least one substance chosen from vitamins, particularly the vitamins of group A (retinol) and group C and derivatives thereof such as the esters, especially the palmitates and propionates, tocopherols, xanthines, particularly caffeine or theophylline, retinoids, particularly vitamin A acid, extracts of Centella asiatica, Asiatic and madecassic acids and glycosylated derivatives thereof such as asiasticoside or madecassoside, extracts of Siegesbeckia orientalis, extracts of Commiphora mukl and extracts of

Eriobotrya japonica, cosmetically acceptable silicon derivatives such as polysiloxanes, silanols and silicones, C_3 –C_{12} aliphatic alpha-keto acids, particularly pyruvic acid, C_2 –C_{12} aliphatic alpha-hydroxy acids, particularly citric acid, glycolic acid, malic acid and lactic acid, lipoic acid, amino acids, particularly arginine, citrulline and threonine, ceramides, glycoceramides, sphinogosine derivative, particularly type II and III ceramides, phospholipids, forskolin and derivatives thereof, extracts of Coleus, extracts of Tephrosia, elastase inhibitors, particularly ellagic acid and soya peptides, collagenase inhibitors, particularly plant peptides and extracts such as extracts of roots of Coptidis and extracts of roots of Scutellaria baicalensis Georgi, flavonoids such as wogonin, baicalin and abaicalein, aqueous-ethanolic extracts of leaves of Ginkgo biloba, Mosla chinensis, Salvia officinalis and Cinnamommum cassia, catechuic extracts of Camellia sinensis and aqueous extracts of bean shells of Theobroma cacao, anti-inflammatories, particularly phospholipase A2 inhibitors, soothing agents, particularly extracts of liquorice, glycyrrhetinic acid and ammonium glycyrrhizinate, hydrating agents, particularly polypols, propylene glycol, butylenes glycol, glycerol and hyaluronic acid, agents for combating stretch marks, particularly extracts of horse chestnut and escin, agents for protecting or improving the microcirculation, particularly bioflavonoids from Ginkgo biloba, isodon, extracts if Ami visnaga, visnadine and ruscogenin, free radical inhibitors, particularly polyphenols such as PCO (procyanidolic oligomers) and derivatives thereof and plant extracts, particularly extracts of Curuma longa, antiseborrhea agents, such as a 5-alpha-reductase inhibitor, particularly an extract of Pygeum africanum, and stimulants of the microcirculation of the blood, such as cepharanthine and methyl nicotinate.

[0047] The compositions according to the invention can advantageously contain substances for protecting the skin from the harmful effects of the sun, such as solar filters, individually or in combination, especially UV A filters and UV B filters, particularly titanium oxides and zinc oxides, oxybenzone, Parsol MCX, Parsol 1789 and filters of vegetable origin, substances for limiting the damage caused to the DNA, particularly those for limiting the formation of thymine dimmers, such as ascorbic acid and derivatives thereof and/or Photonyl.RTM., and substances for contributing to the elimination of liver spots, such as inhibitors of melamin or tyrosinase synthesis.

[0048] The invention also relates to the method further comprising: mixing into the platelet composition substantially simultaneously with its topical application to the skin, with one or more of the ingredients selected from thrombin, epinephrine, collagen, calcium salts, and pH adjusting agents. Also useful are materials to promote degranulation or preserve platelets, additional growth factors or growth factor inhibitors, small molecule pharmaceuticals such as NSAIDS, steroids, and anti-infective agents.

[0049] In yet another aspect, the invention relates to a dermatological composition comprising: platelet releasate wherein the composition is at a pH greater than or equal to physiological pH, and wherein the composition comprises substantially no unactivated platelets.

[0050] PRP is a concentration of platelets greater than the peripheral blood concentration suspended in a solution of plasma, with typical platelet counts ranging from 500,000 to 1,200,000 per cubic millimeter, or even more. PRP is formed from the concentration of platelets from whole blood, and may be obtained using autologous, allogenic, or pooled sources of platelets and/or plasma. PRP may be formed from a variety of animal sources, including human sources.

[0051] Platelets are cytoplasmic portions of marrow megakaryocytes. They have no nucleus for replication; the expected lifetime of a platelet is some five to nine days. Platelets are involved in the hemostatic process and release several initiators of the coagulation cascade. Platelets also release cytokines involved with initiating wound healing. The cytokines are stored in alpha granules in platelets. In response to platelet to platelet aggregation or platelet to connective tissue contact, as would be expected in injury or surgery, the cell membrane of the platelet is "activated" to secrete the contents of the alpha granules. The alpha granules release cytokines via active secretion through the platelet cell membrane as histones and carbohydrate side chains are added to the protein backbone to form the complete cytokine. Platelet disruption or fragmentation, therefore, does not result in release of the complete cytokine.

[0052] A wide variety of cytokines are released by activated platelets. Platelet derived growth factor (PDGF), transforming growth factor-beta (TGF-b), platelet-derived angiogenesis factor (PDAF) and platelet derived endothelial cell growth factor (PD-ECGF) and insulin-like growth factor (IGF) are among the cytokines

released by degranulating platelets. These cytokines serve a number of different functions in the healing process, including helping to stimulate cell division at an injury site. They also work as powerful chemotactic factors for mesenchymal cells, monocytes and fibroblasts, among others.

[0053] Historically, PRP has been used to form a fibrin tissue adhesive through activation of the PRP using thrombin and calcium, as disclosed in U.S. Patents 5,165,938 to Knighton, and 5,599,558 to Gordinier et al., incorporated in their entirety by reference herein. Activation results in release of the various cytokines and also creates a clotting reaction within various constituents of the plasma fraction. The clotting reaction rapidly forms a platelet gel (PG) which can be applied to various wound surfaces for purposes of hemostasis, sealing, and adhesion.

[0054] In another embodiment, the inventive platelet composition may comprise releasate from platelets, in addition to platelets themselves. The releasate comprises the various cytokines released by degranulating platelets upon activation. Many activators of platelets exist; these include calcium ions, thrombin, collagen, epinephrine, and adenosine diphosphate. Releasates according to the invention may be prepared according to conventional methods, including those methods described in U.S. Patents 5,165,938 to Knighton, and 5,599,558 to Gordinier et al. The releasates alone or in a dermatologically acceptable carrier may be topically applied and/or injected into the skin.

[0055] One disadvantage of conventional releasate strategies associated with the use of PRP as PG is the use of thrombin as a preferred activator. In particular, much thrombin used in PG is bovine thrombin, which can create problems due to contamination issues regarding prions which cause Creutzfeldt-Jakob disease. Many bovine materials are suspect due to possible prion contamination, and so use of bovine thrombin is disfavored. Human pooled thrombin is likewise disfavored due to the potential of contamination with various infectious agents such as viruses, prions, bacteria and the like. Recombinant human thrombin might also be used, but may be expensive. Any of the platelets, fibroblast cells, thromin, or formulations of the invention or components thereof may be tested for the presence of prions using assays known in the art such as disclosed in U.S. Patents 6,620,629 issued

September 16, 2003 and; 6,221,614; 6,617,119 issued September 9, 2003; and 5,891,641.

[0056] It is a particular advantage of the present invention that exogenous or extra activators need not be administered to a patient. Collagen, a major component of connective tissues, is a strong activator of platelets. Thus, when the inventive platelet composition is administered to skin, platelets in the platelet composition may bind to the collagen and then be activated. This reduces or eliminates the need for administering an exogenous activator such as thrombin. The disadvantages of thrombin use have been noted above. Other strong activators, such as calcium ions, can cause severe pain, unintentional clotting, and other undesirable side effects. Thus, in an embodiment of the invention, no or substantially no exogenous activator is present or added as part of the inventive platelet composition, or is used in the preparation of the inventive platelet composition. Of course, exogenous activators may still be employed if a physician determines that they are medically necessary or desirable. Thus, the composition of the invention may consist only of platelets as the active ingredient.

[0057] The platelet composition may be prepared using any conventional method of isolating platelets from whole blood or platelet-containing blood fractions. These include centrifugal methods, filtration, affinity columns, and the like. If the platelet composition comprises PRP, then conventional methods of obtaining PRP, such as those disclosed in U.S. Patents 5,585,007 and 5,788,662 both to Antanavich et al., incorporated herein by reference in their entirety, may be utilized.

[0058] Adjusting the pH of platelet compositions has been used to prolong the storage time of unactivated platelets, as disclosed in U.S. Patents 5,147,776 to Koerner, Jr. and 5,474,891 to Murphy, incorporated by reference herein. pH may be adjusted using a variety of pH adjusting agents, which are preferably physiologically tolerated buffers, but may also include other agents that modify PRP pH including agents that modify lactic acid production by stored platelets. Especially useful are those pH adjusting agents that result in the pH of the platelet composition becoming greater than or equal to physiological pH. In an embodiment, the pH adjustment agent comprises sodium bicarbonate. Physiological pH, for the purposes of this invention, may be defined as being a pH ranging from about 7.35 to about 7.45. pH adjusting agents useful in the practice of

this invention include bicarbonate buffers (such as sodium bicarbonate), calcium gluconate, choline chloride, dextrose (d-glucose), ethylenebis(oxyethylenenitrilo)tetraacetic acid (EGTA), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), maleic acid, 4-morpholinepropanesulfonic acid (MOPS), 1,4-piperazinebis(ethanesulfonic acid) (PIPES), sucrose, N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), tris(hydroxymethyl)aminomethane (TRIS BASE), tris(hydroxymethyl)aminomethane hydrochloride (TRIS.HCl), and urea. In a preferable embodiment, the pH adjusting agent is a bicarbonate buffer, more preferably, sodium bicarbonate.

PLATELET ALLOIMMUNIZATION

[0059] Platelets present a variety of antigens, including HLA and platelet-specific antigens. Patients transfused with platelets which are not their own often develop HLA antibodies. The patient may become refractory to all but HLA-matched platelets. When platelets are transfused to a patient with an antibody specific for an expressed antigen, the survival time of the transfused platelets may be markedly shortened. Nonimmune events may also contribute to reduced platelet survival. It is possible to distinguish immune from nonimmune platelet refractoriness by assessing platelet recovery soon after infusion, i.e., 10 - 60 minutes postinfusion platelet increment. In immune refractory states secondary to serologic incompatibility, there is poor recovery in the early postinfusion interval. In nonimmune mechanisms, i.e., splenomegaly, sepsis, fever, intravascular devices, and DIC, platelet recovery within 1 hour of infusion may be adequate while long-term survival (i.e., 24-hour survival) is reduced. Serologic tests may be helpful in selecting platelets with acceptable survival. In accordance with the present invention the platelets are preferably taken from the same patient they will be used to treat. In a similar manner the platelet releasate or any portion thereof is taken from the same patient treated with the formulation. Alternatively, the patient is treated with platelets, platelet releasate and portions thereof extracted from a donor patient tested for and found to have a close serologic match with the patient being treated.

CELL CULTURES

[0060] The cell cultures of the present invention involved the use of PRP and, for example may use PRP from the same patient the cells (e.g. fibroblast cells) being cultured were obtained from.

[0061] Example 5 below shows the cell culture with PRP therein and Example 6 shows the cell culture with three different concentrations of platelet releasate therein. The platelets may be treated in any manner to open the platelets or allow the releasate to escape. The treatment may be with an energy wave (e.g. ultra sound), agitation, temperature (heating/cooling- freezing/thawing), and chemical treatments or any combination thereof.

[0062] Many kinds of cells can be grown in culture, provided that suitable nutrients and other conditions for growth are supplied. Thus, since 1907 when Harrison noticed that nerve tissue explanted from frog embryos into dishes under clotted frog lymph developed axonal processes, scientists have made copious use of cultured tissues and cells from a variety of sources. Such cultures have been used to study genetic, physiological, and other phenomena, as well as to manufacture certain macromolecules using various fermentation techniques known in the art.

[0063] In studies of mammalian cell biology, cell cultures derived from lymph nodes, muscle, connective tissue, kidney, dermis and other tissue sources have been used. Generally speaking, the tissue sources that have been most susceptible to the preparation of cell cultures for studies are derivatives of the ancestor mesodermal cells of early development. Tissues that are the progeny of the ancestor endodermal and ectodermal cells have only in recent years become amenable to cell culture, of a limited sort only. The cell types derived from the endoderm and ectoderm of early development include epidermis, hair, nails, brain, nervous system, inner lining of the digestive tract, various glands, and others. Essentially, long-term cultures of normal differentiated human cells, particular certain types of cells, are difficult to obtain. For various types of cartilage cultures see U.S. Patent 5,902,741 issued May 11, 1999.

[0064] The cell-types subjected to a procedure of the present invention are derived from various tissues, can be of human origin or that of any other mammal, and may be of any suitable source, such as fibroblast cells, stem cells, cell from a whole

pancreas, parotid gland, thyroid gland, parathyroid gland, prostate gland, lachrymal gland, cartilage, kidney, inner ear, liver, parathyroid gland, oral mucosa, sweat gland, hair follicle, adrenal cortex, urethra, and bladder, or portions or multiples thereof.

[0065] The tissue is prepared using any suitable method, such as by gently teasing apart the excised tissue or by digestion of excised tissue with collagenase via, for example, perfusion through a duct or simple incubation of, for example, teased tissue in a collagenase-containing buffer of suitable pH and tonic strength. The prepared tissue then is concentrated using suitable methods and materials, such as centrifugation through ficol gradients for concentration (and partial purification). The concentrated tissue then is resuspended into any suitable vessel, such as tissue culture glassware or plasticware. The resuspended material may include whole substructures of the tissue, cells and clusters of cells. For example, such substructures may include fibroblast cells.

[0066] The initial culture of resuspended tissue cells is a primary culture. In the initial culturing of the primary culture, the cells attach and spread on the surface of a suitable culture vessel with concomitant cell division. Subsequent to the initial culture, and usually after the realization of a monolayer of cells in the culture vessel, serially propagated secondary and subsequent cultures are prepared by dissociating the cells of the primary culture and diluting the initial culture or its succeeding cultures into fresh culture vessels, a procedure known in the art as passaging. Such passaging results in an expanded culture of cells of the originating tissue. The cell culture is passaged at suitable intervals, such as about once a week or after about two to about three cell divisions of the cultured cells. Longer intervals of two to three weeks or shorter intervals of two to three days would suffice also. For passaging the cell cultures, a dilution of the cultured cells at a ratio of from about 1:2 to about 1:100 is used. Preferably, a ratio of from about 1:4 to about 1:50 is used. More preferably, a ratio of from about 1:4 to about 1:6 is used.

[0067] The concentrated prepared tissue, which may be in the form of free cells and/or clumps (where the clumps may constitute ordered substructures of the tissue) is resuspended at any suitable initial cell or presumptive cell density. Suitable cell densities range from about 100 cells to about 1000 cells per square centimeter of

surface area of the culture vessel. For useful vessels see U.S. Patent 5,274,084 issued December 21, 1993 and patents and publications cited therein.

[0068] Basal media that may be used include those commercially available from Sigma Chemical Co., Life Technologies, Inc., or BioWhittaker Co. Any basal medium may be used provided that at least magnesium ion, calcium ion, zinc ion, bicarbonate ion, potassium ion, and sugar levels can be manipulated to a lower or higher concentration in the resultant medium; in particular, the magnesium ion, calcium ion, bicarbonate ion, and D-glucose levels are required at a lower concentration, zinc ion is required at the same or higher concentration, and potassium ion is required at the same or lower concentration than is usual in standard basal media.

[0069] Preferred levels of magnesium ion, as contributed by suitable magnesium salts, such as $MgSO_4$ .multidot.$7H_2O$ and $MgCl_2$ .multidot.$6H_2O$, are between 60 and 240 mg/L; more preferred levels of magnesium salts are between 100 and 150 mg/L. Preferred levels of calcium ion, as contributed by suitable calcium salts, such as $CaCl_2$ .multidot.$2H_2O$, are between 25 and 200 mg/L; more preferred levels of calcium ion are between 40 and 125 mg/L. Preferred levels of zinc ion, as contributed by suitable zinc salts, such as $ZnSO_4$ .multidot.$7H_2O$, are between 0.1 and 0.5 mg/L; more preferred levels of zinc ion are between 0.12 and 0.40 mg/L; yet more preferred levels of zinc ion are between 0.15 and 0.20 mg/L. Preferred levels of ascorbic acid are between 30 and 125 mg/L; more preferred levels of ascorbic acid are between 40 and 100 mg/L. Preferred levels of bicarbonate ion, as contributed by suitable bicarbonate salts, such as sodium bicarbonate, are between 175 and 700 mg/L; more preferred levels of bicarbonate ion are between 300 and 400 mg/L. Preferred levels of potassium ion, as contributed by suitable potassium salts, such as potassium chloride, are between 100 and 400 mg/L; preferred levels of potassium ion are between 200 and 325 mg/L; most preferred levels of potassium ion are between 210 and 250 mg/L. Preferred levels of sugar, as contributed by a suitable sugar, such as D-glucose, are between 400 and 1800 mg/L; more preferred levels of sugar are between 600 and 1200 mg/L; most preferred levels of sugar are between 800 and 1000 mg/L. Preferred levels of human placental lactogen are between 3 and 15 .mu.g/ml; more preferred levels of human placental lactogen are between 4 and 13 .mu.g/ml; most preferred levels of human placental lactogen are

between 8 and 12 .mu.g/ml. Preferred levels of insulin, as contributed by a suitable naturally-isolated, clonally-derived, or synthesized insulin, such as isolated bovine sodium-insulin, are between 50 and 20,000 ng/ml; more preferred levels of insulin are between 100 and 10,000 ng/ml; most preferred levels of insulin are between 500 and 5,000 ng/ml. (See U.S. Patent 6,008,047 issued December 28, 1999)

[0070] The cells such as fibroblasts and keratinocytes used in accordance with the present invention may be either autogenic or allogenic. The use of allogenic cells enables the production and storage of the living skin equivalent of the present invention thereby avoiding delays in procuring grafts for the treatment of wounds. Both cell types, keratinocytes and fibroblasts could be stored frozen for months as single cell suspensions, using published methods. After thawing these cells should maintain their viability and grow readily in culture. (See U.S. Patent 6,039,760 issued March 21, 2000)

TOPICAL FORMULATIONS

[0071] The PRP obtained can be dispersed in, mixed with or combined in any fashion with a dermatologically acceptable carrier to create a topical formulation. The formulation may be an ointment, cream, lotion, oil or the like that can be placed on the skin of a human. The carrier may be comprised of natural, refined or synthetic oils. The carrier may be derived from a liquid petroleum gelled by the addition of a polyethylene resin. Composition based on animal fats, and/or vegetable oils may be used including lard, benzoinated lard, olive oil, cottonseed oil and the like. Examples of topical formulations are described and disclosed in publications such as Remington's Pharmaceutical Sciences, (18^{th} Ed.) Mack Publishing, Co. 1990. Such formulations may comprise a preservative and bacterialcidal and/or bacterialstatic compounds as well as perfumes and coloring agents.

[0072] The topical formulations may have a buffer adder to the PRP or have the buffer in the carrier. The pH of the formulation should be balanced to obtain a pH close to physiological pH e.g. about 7.4 ± 10% or ± 5%, or 7.2 to 7.6.

[0073] The presence of other active ingredients may require a different overall pH for the formulation as some active ingredients require a particular pH range. The relesate, platelets and/or the platelets and releasate may be combined with the

carrier over a wide range of concentrations, e.g. 1%, 10%, 25%, 50%, 75%, 90%, 95%, 99% carrier with the remainder being PRP, platelets, platelet releaseate or combinations thereof with or without an additional active ingredient.

TRANSDERMAL PATCH

[0074] FIG. 4 shows a first type of transdermal patch 1 which includes an impermeable support film 2 on which a matrix 3 is arranged. The active substance which is a blood cocentrate such as PRP or platelet releasate, and/or combinations thereof, is dissolved and/or dispersed in the matrix 3 which serves as a reservoir. The matrix 3, on the opposite side to the impermeable support film 2, is covered by a membrane 4 permeable to the blood concentrate, which regulates the cross-flow. This membrane may not be necessary if the degree of permeation of the blood cocentrate such as PRP or platelet releasate in the skin does not exceed the values which might cause side effects.

[0075] The degree of diffusion of the active substance (e.g. blood cocentrate such as PRP or platelet releasate) will also depend on the permeation activators, solubilizers, etc. On the free side of the permeable membrane 4 there is a layer of a contact glue 5 (adhesive layer), protected by a release strip 6. During the use of the transdermal patch the release strip 6 is pulled off and the patch is positioned on the desired part of the patient's body, exerting slight pressure. After a "start" phase the flow reaches a constant "saturation" value.

[0076] The patch 1' of (FIG. 5), a matrix or "reservoir" 3, in which the active substance (e.g. blood concentrate such as PRP or platelet releasate) is dissolved and/or dispersed, is applied on the impermeable support film 2'. In the present case the membrane permeable to the active substance, which is used to modify the cross-flow, is missing. The matrix 3' therefore comes into direct contact with the epidermis. The glue 5' is located around the edge of the patch, like an adhesive ring. Everything is protected on the free side by a single release strip 6', which is removable, as for the embodiment of Figure 4.

[0077] The use of the "device" is as follows: the release strip 6' is pulled off and the device is positioned on the desired part of the patient's body, exerting slight pressure.

[0078] The embodiment of Figure 5 can be adopted in particular if the active principle interacts in an unwanted manner with the adhesive, as a result of which it is not possible to mix the adhesive 5' and the active principles in the matrix 3'.

[0079] The pharmacological dose (e.g. blood concentrate such as PRP or platelet releasate) may be placed directly, dissolved or dispersed, into the glue, which thus also becomes a "reservoir" which may be arranged in a layer on a permeable support film. A person skilled in the art reading this disclosure will be able to modify the shape and/or structure of the patch, achieving the best result based on the therapy chosen and the site of application; or on other factors.

[0080] The differences in the structure and shape of the patch (rectangular or anatomical) may be due:

[0081] to the area of skin being treated;

[0082] to the interactions which may exist between the active principle, the glue (different types of adhesive can be used simultaneously), the support material, and other materials such as excipients, stabilizers, etc.;

[0083] to better stability on the chosen site of application;

[0084] to the dosage (the area of the patch must also increase for a higher dosage).

METHOD OF MANUFACTURE OF THE TRANSDERMAL PATCH

[0085] 1. The active principle (e.g. blood concentrate such as PRP or platelet releasate) is incorporated simultaneously with the other components (stabilizer, permeation activators, etc.) in the hot adhesive solution and homogenized by stirring, until the liquid adhesive matrix or "reservoir" is obtained;

[0086] 2. the liquid matrix is cooled, and acquires a "stringy" consistency;

[0087] 3. the process for layering of the adhesive matrix on the support is carried out using a layering machine which is continuously connected with a drying machine, in the following phases:

[0088] the blade of a knife is mounted across the entire width of the conveyor belt of the layering machine on which the release strip is securely positioned;

[0089] the "stringy" adhesive matrix is poured in front of the blade, which, as the conveyor belt advances, distributes a uniform layer (layering) of adhesive matrix on the release strip;

[0090] the thickness of the layer is mainly determined by the distance between the edge of the knife blade and the release strip running beneath it;

[0091] the release strip, carrying the adhesive matrix, rotates inside the drying machine, in which the adhesive matrix is solidified by evaporation of the solvent, which is achieved by gradually increasing the temperature and the "ventilation", as shown in the following Table I.

TABLE I

Drying phase	Time (in minutes)	T°C	Vent (rpm)
1	15	40	700
2	20	55	1000
3	25	70	1200

[0092] The process described allows elimination of the solvent, preventing it from being occluded by the rapid formation of a surface crust.

[0093] When the adhesive matrix has dried, the support film (backing) is applied. This phase, called "lamination", ends the process.

[0094] The adhesive should be inert and permeable to the active compound (e.g. blood concentrate such as PRP or platelet releasate), and the adhesive properties of which (cohesion, adhesion and interlacing) are not adversely affected by the blood concentrate such as PRP or platelet releasate itself and/or by excipients or any other material added.

COMPOSITION OF THE PATCH

[0095] Adhesive Matrix: Formulation

[0096] active principle: blood concentrate such as PRP or platelet releasate;

[0097] antioxidant: sodium metabisulphice, EDTA disodium salt;

[0098] solubilizing agent: a glycol;

[0099] permeation activator: fatty acids;

[00100] acrylic resin to improve the cohesive strength: cationic copolymers based on dimethylaminoethylmethacrylate and methacrylic esters;

[00101] cellulose derivatives to improve the cohesive strength: ethylcellulose;

[00102] surfactant: SDS (sodium dodecylsulphate);

[00103] pressure contact adhesive: mixture of two adhesives, A and B, in which A is a non-self-bonding acrylic contact adhesive of medium molecular weight with a high interlacing index, with a skin irritation index of 0.20, classified as "minimally irritating", using 100% ethyl acetate as solvent; and B is a self-bonding acrylic adhesive with a high molecular weight, with moderate interlacing, with a skin irritation index of 0, classified as "non-irritating", using a mixture of ethyl acetate, isopropanol, hexane and toluene as solvent.

RELEASE STRIP

[00104] The release strip is a polyester film laminated with silicone on one side (that opposite of the adhesive matrix). The thickness is approximately 125 microns.

[00105] "Backing"

[00106] The "backing" is a laminated polyester film which is clear and occlusive with a themoweldable layer. The total thickness is approximately 51 microns.

[00107] Quantity of Active Principle

[00108] The quantity of blood concentrate such as PRP or platelet releasate is 5% by weight of the adhesive matrix and corresponds to 5 mg/sq cm. The major part of the blood concentrate such as PRP or platelet releasate is dispersed in the matrix. A minor part is dissolved in the matrix. The active component e.g. blood concentrate such as PRP or platelet releasate dispersed in the matrix acts as a "reservoir", while the active component available for release and permeation is the dissolved active component.

[00109] Prophetic examples of the patch based with the active component (e.g. blood concentrate such as PRP or platelet releasate) are now given.

[00110] Two batches of patches of differentiated formulation containing active components are prepared for this purpose.

EXAMPLE A

[00111]	1) platelet releasate	2.20%
[00112]	2) Solubilizing agent	4.00%

[00113]	3) Acrylic resin	29.00%
[00114]	4) Fatty acid 1	3.20%
[00115]	5) Fatty acid 2	1.60%
[00116]	6) Pressure-sensitive adhesive	60.0%

EXAMPLE B

[00117]	1) PRP	4.99%
[00118]	2) EDTA	0.025%
[00119]	3) Solubilizing agent	9.96%
[00120]	4) Fatty acid 1	7.96%
[00121]	5) Fatty acid 2	3.97%
[00122]	6) Acrylic resin	1.99%
[00123]	7) Cellulose derivative	0.25%
[00124]	8) Surfactant	20.4%
[00125]	9) Pressure-sensitive adhesive	50.455%

PATCH DEVOID OF DRUG

[00126] There are a wide range of transdermal patches known in the art and, in general, those patches can be modified to incorporate a platelet releasate formulation of the invention. However, in an embodiment of the invention the platelet releasate is autologous to the patient being treated. To achieve such the patch can be produced without a drug (e.g. without platelet releasate) in it. The patch may have a single or multiple compartments which can be filled with the patient's own platelet releasate prior to placing the patch on the patient's skin. This can be accomplished in a number of different ways. For example, the patch may comprise a compartment which is empty or comprises a gauze, matrix or like material which readily absorbs a liquid such as releasate formulation of the invention.

[00127] The compartment may be covered by a lid which has a resealable adhesive around its edges. This makes it possible for the lid to be opened, liquid formulation

is added to the container and the lid resealed. An alternative is to have a wall or portion of a wall that is comprised of a material that is self sealing when punctured with a hollow cylinder such as a hollow needle used to inject a formulation of the invention into the compartment.

[00128] In accordance with this embodiment of the invention blood is extracted from a patient and platelets of the blood concentrated. The concentrated platelets are subjected to treatment such as sonification to create a platelet releasate. The releasate is formulated to adjust the pH. The pH balanced, liquid, flowable formulation is placed in the compartment. The patient, which may be the patient from which the blood was taken, applies the patch and releasate formulation is administered transdermally. The patch may be in any shape and may, for example, be in the shape of a 3-dimensional mask shaped to fit the face of the patient or a portion of the face of the patient such as over the patient's forhead and around the patient's eyes where wrinkles are most prominent. The patch can be repeatedly applied, night after night, and worn by the patient during sleep and/or just prior to sleep. The releasate is shown to promote the growth of fibroblast cells in the cell culture of Examples 6 and 7 and fibroblast cells are essential for the young healthy appearance of skin.

[00129] An individualized transdermal patch of the invention can also be used in wound healing. The patch is prepared in a manner as indicated above and applied to a wound. The wound may also be treated with other compounds such as antibodies to aid in the treatment of infection.

[00130] Transdermal patches generally have configurations similar to those shown in Figures 4 and 5. However, in an aspect of the present invention the various layer of skin and cells just below the skin are being treated to provide an improved appearance to the skin and particularly skin on the face. This may be obtained using a patch as shown in Figure 6. The patch 7 has a two dimensional shape which covers the user's forehead with sections 8 and 9 separated by a border 10. The area under the user's eyes exposed by opening 11 and 12 is covered by section 13 which include a 3-dimensional raised area 14 to accommodate the user's nose. There may be openings 15 and 16 to allow the user's eyebrows the protrude.

[00131] A cross-section of the patch 7 is shown in Figure 7. The polymeric backing layer 17 may be skin colored on its surface 18. The layer 17 covers a compartment

or reservoir 19 which holds to drug formulation such as the platelet releasate. The formulation is allowed to flow out through a membrane layer 20 which may be a semi-permeable membrane, or a gauze. The layer 21 is a removable liner which when peeled away allows formulation to seep from the reservoir 19 through the member 20 to and through the skin of the user. The patch 7 may be held in palce by adhesive at edges 22 and 23.

[00132] The layer 17 includes an area 24 as shown in Figure 7 which may be comprised of a self-sealing material such as a number of polymers which when permeated by a hollow needle will re-seal itself. The formulation individualized for the patient can be injected through the area 24. Figure 6 shows area 24 and other areas 26, 27 and 28. Areas 26 is the same as area 24 but allow for formulation to be added to section 13 of the patch 7. Areas 27 and 28 respectively allow for formulation to be added to sections 13 and 9. The area 27 and 28 can be opened and peeled back as shown with area 28 and resealed as shown in area 27 such as with an adhesive which can be repeatedly opened and resealed. In the manner the patch can be individualized for the patient (e.g. with releasate obtained from the blood of the patient being treated with the patch) and area of the patch can be loaded with different formulations useful in treating specific areas of the face. For example, sensitive areas under the eye in section 13 might be too sensitive for the use of retinol whereas the forehead sections 8 and 9 could be supplemented with retinol.

[00133] Those skilled in the art reading this disclosure will understand that the patch 7 may cover the user's entire face (with openings for nose, mouth and eyes) or any portion of the face. Further, the patch may be divided into any number of sections with any number of areas to add formulation. In the method of the invention a face patch as shown in Figures 6 and 7 is repeatedly applied each night. Adjustments in the amount of active ingredient and type of active ingredient added to the patch as a whole or to any particular section of the patch can be adjusted each day or each week or month or as needed depending on observed results obtained.

EXAMPLES

[00134] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Example 1

[00135] PRP was prepared using a centrifuge unit made by Harvest (Plymouth, MA). (Similar units are available as The Biomet GPS system, the Depuy Symphony machine and the Medtronic Magellan machine.) Approximately 55 cc of blood was drawn from the patient using a standard sterile syringe, combined with 5 cc of a citrate dextrose solution for anticoagulation, and then spun down to isolate the platelets according to the manufacturer's protocol. These platelets were then resuspended in approximately 3 cc of plasma. The resulting platelet rich plasma solution (PRP) was quite acidic and was neutralized with using approximately 0.05 cc of an 8.4% sodium bicarbonate buffer per cc of PRP under sterile conditions to approximately physiologic pH of 7.4. The PRP was not activated through addition of exogenous activators. This PRP composition is referred to herein as autologous platelet extract (APEX).

Example 2

[00136] Fifty cc of whole blood is drawn from a patient, and then prepared according to the method of Knighton, U.S. Patent 5.165,938, column 3. The PRP is activated according to Knighton using recombinant human thombin. The degranulated platelets are spun down and the releasate containing supernatant is recovered. The releasate may be optionally pH adjusted to a pH of 7.4 using sodium bicarbonate buffer.

Example 3

[00137] Thirty ml of whole blood were drawn from a patient. A platelet composition was prepared according to Example 1 of U.S. Patent 5,510,102 to Cochrum, incorporated herein by reference in its entirety, except that no alginate is added to the platelet composition.

Example 4: Cell Cultures of Any Tissue

[00138] A researcher or clinician wishes to grow a cell culture of either fibroblasts or osteoarthritic cartilage cells. Using the technique of Example 1, an autologous platelet extract (APEX) is obtained and buffered to physiologic pH.

[00139] The cells are then isolated and grown in a media rich in the APEX in various conditions and dilutions. The APEX promotes cell differentiation and production of proteins such as collagen. The APEX may augment or promote the ability of the cells to transform into normal cells. Without intending to be limited by theory, it is hypothesized the APEX may convert the osteoarthritic cartilage cells to a more functional cell line that is reinjected into a diseased or injured joint. Alternatively, the APEX is directly introduced into an osteoarthritic joint to reverse the course of the disease. This is done under local anesthesia in a sterile manner.

Example 5: Human Fibroblast Proliferation in Buffered Platelet Rich Plasma

[00140] Platelet rich plasma has been used to augment bone grafting and to help accelerate or initiate wound healing. Fibroblasts are important components of the wound healing process. This example shows that human fibroblast cells will proliferate more in fetal bovine serum that has been augmented with a proprietary formulation of buffered platelet rich plasma.

[00141] Human fibroblasts were isolated and then put into culture with 10% fetal bovine serum that had been augmented with a proprietary formulation of buffered platelet rich plasma (Group 1) or in 10% fetal bovine serum alone (Group 2). Initial cell counts were 25,000 in both groups.

[00142] Seven days after initiating the culture experiment, the cells in each group were counted. The average total cell count in Group 1 (buffered PRP added) was 1,235,000. The average total cell count in Group 2 (No PRP) was 443,000. The

group that was augmented with the buffered platelet rich plasma of the invention had 2.8 times the proliferation of the control group at seven days. (See Figure 1)

[00143] Buffered platelet rich plasma augments human fibroblast proliferation when compared to the use of fetal bovine serum alone. This has significant implications for the use of buffered platelet rich plasma for either acute or chronic wound healing.

Example 6

Human Fibroblast Proliferation in Sonicated Platelet Rich Plasma

[00144] Human fibroblasts were isolated and then put into four different cultures. Three of the cultures comprised 10% fetal bovine serum that had been augmented with 9uL, 46uL, and 95uL of buffered and sonicated platelet rich plasma. The fourth served as the control and was comprised of 10% fetal bovine serum. Initial cell counts were 20,000 in both groups. Variable doses of the sonicated PRP (sPRP) were seeded with cells.

[00145] Four days after initiating the culture experiment, the cells in each of the four groups were counted and the results are shown in Figure 2. The cell count in the control group (No PRP) was 180,000 cells. The cell counts in the sonicated PRP group were as follows: 496,000 (9 uL dose of sPRP), 592,000 (46uL dose of sPRP) and 303,000 (95uL dose of sPRP).

[00146] This experiment shows that buffered, and sonicated platelet rich plasma augments human fibroblast proliferation when compared to the use of fetal bovine serum alone.

Example 7

Human Fibroblast Proliferation in Sonicated Platelet Rich Plasma

[00147] Human fibroblasts were isolated and then put into twor different cultures. One of the cultures comprised 10% fetal bovine serum that had been augmented with buffered and sonicated platelet rich plasma. The other served as the control and was comprised of 10% fetal bovine serum. Initial cell counts were 20,000 in both groups.

[00148] Seven days after initiating the culture experiment, the cells in each of the two groups were counted and the results are shown in Figure 3. The cell count in the control group (No PRP) was 183,600 cells. The cell count in the sonicated PRP group was 924,800 cells.

[00149] This experiment shows that buffered, and sonicated platelet rich plasma augments human fibroblast proliferation when compared to the use of fetal bovine serum alone. These results show the ability of the platelet releasate to promote cell growth and in particular fibroblast cells which are essential to firm, young looking skin.

[00150] The preceding merely illustrates the principles of the invention. It will be appreciated that those skilled in the art will be able to devise various arrangements which, although not explicitly described or shown herein, embody the principles of the invention and are included within its spirit and scope. Furthermore, all examples and conditional language recited herein are principally intended to aid the reader in understanding the principles of the invention and the concepts contributed by the inventors to furthering the art, and are to be construed as being without limitation to such specifically recited examples and conditions. Moreover, all statements herein reciting principles, aspects, and embodiments of the invention as well as specific examples thereof, are intended to encompass both structural and functional equivalents thereof. Additionally, it is intended that such equivalents include both currently known equivalents and equivalents developed in the future, i.e., any elements developed that perform the same function, regardless of structure. The scope of the present invention, therefore, is not intended to be limited to the exemplary embodiments shown and described herein. Rather, the scope and spirit of present invention is embodied by the appended claims.

CLAIMS

That which is claimed is:

1. A transdermal patch comprising:
at least one support film;
an intermediate matrix comprising a pharmaceutical composition; and
a protective release strip,
wherein the pharmaceutical composition comprises a blood concentrate in a pharmaceutically acceptable and effective amount.

2. A transdermal patch according to claim 1, further comprising:
a component chosen from stabilizers, solubilizers, surfactants, and permeation activators to facilitate the passage of the blood concentrate through skin.

3. A transdermal patch according to claim 2, wherein said stabilizers are antioxidant substances, the solubilizer is a glycol and the permeation activators are chosen from a fatty acid and an alcohol.

4. A transdermal patch according to claim 1, wherein the blood concentrate is platelets subjected to sonification and the matrix is comprised of collagen.

5. A transdermal patch according to claim 1, wherein the pharmaceutical composition further comprises retinol.

6. A transdermal patch according to claim 2, wherein the pharmaceutical composition further comprises a vitamin A acid.

7. A transdermal patch according to claim 4, further comprising:
a buffer in an amount to adjust the pH of the platelets to about 7.4 ± 5%.

8. A transdermal patch according to claim 1, further comprising:
an acrylic resin to improve the cohesive strength, cellulose derivatives to improve the cohesive strength, and a mixture of pressure contact adhesives.

9. A transdermal patch according to claim 2, further comprising:

an adhesive matrix containing an acrylic resin, cellulose derivatives to improve the cohesive strength, and a mixture of pressure contact adhesives.

10. A transdermal patch according to claim 2, wherein said surfactant is sodium dodecyl sulfate.

11. A transdermal patch according to claim 1, wherein said blood concentrate is platelet-rich plasma.

12. A transdermal patch according to claim 1, wherein said blood concentrate is platelet releasate.

13. A transdermal patch according to claim 1, wherein said blood concentrate is platelets and platelet releasate.

14. The transdermal delivery system of claim 1, wherein said solvent is an alcohol or water.

15. The transdermal delivery system of claim 14, wherein said alcohol is selected from the group consisting of isopropanol, ethanol, methanol and mixtures thereof.

16. The transdermal delivery system of claim 1, wherein said solvent is selected from the group consisting of olive oil, peanut oil, soybean oil, monoi oil, macadamia oil sunflower oil, glycerin, cetyl alcohol and propylene glycol.

17. The transdermal delivery system of claim 1, wherein the amount of said solvent ranges from about 1% to 60% by weight.

PATIENT INDIVIDUALZED TRANSDERMAL PATCH

ABSTRACT OF THE DISCLOSURE

[00151] A transdermal drug delivery system comprises a laminate composite with a single or a plurality of compartments for a unit dose of individualized active ingredient to be transdermally administered. The assembly is adhesively secured to the skin of a patient. Individual seals are provided for adding the active component resealably enclosing the active component in each of the reservoirs. The active component may be any isolated component of the patient's blood including isolated platelets which are sonicated and formulated with an adjusted pH. The patch is applied to the patient's skin, such as wrinkled skin on the face to provide an improved appearance to the skin.

F:\DOCUMENT\AMBI\006PRV\AMBI-006PRV - appln.doc

FIG. 1

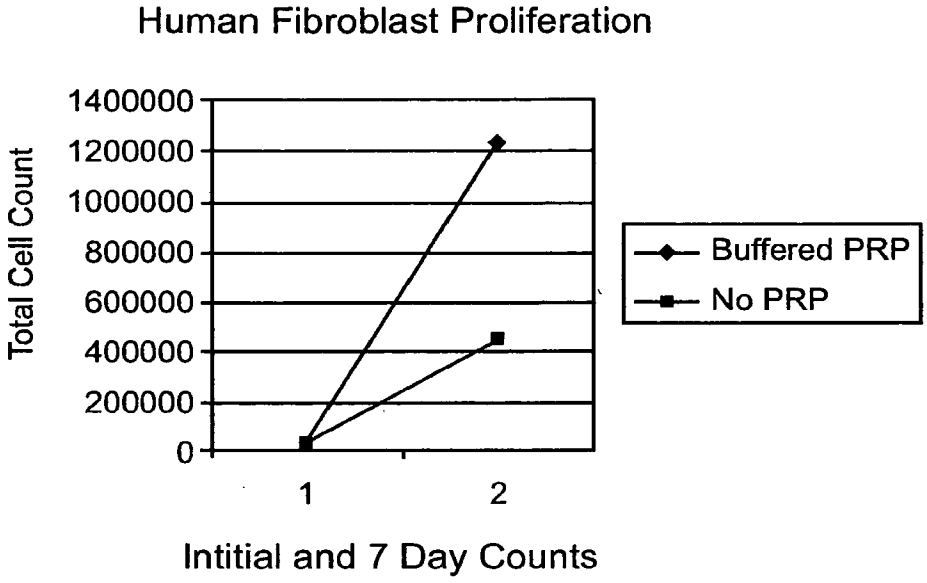

Human Fibroblast Proliferation
Total Cell Count
1400000
1200000
1000000
800000
600000
400000
200000
0
1
2
Intitial and 7 Day Counts
Buffered PRP
No PRP

FIG. 2

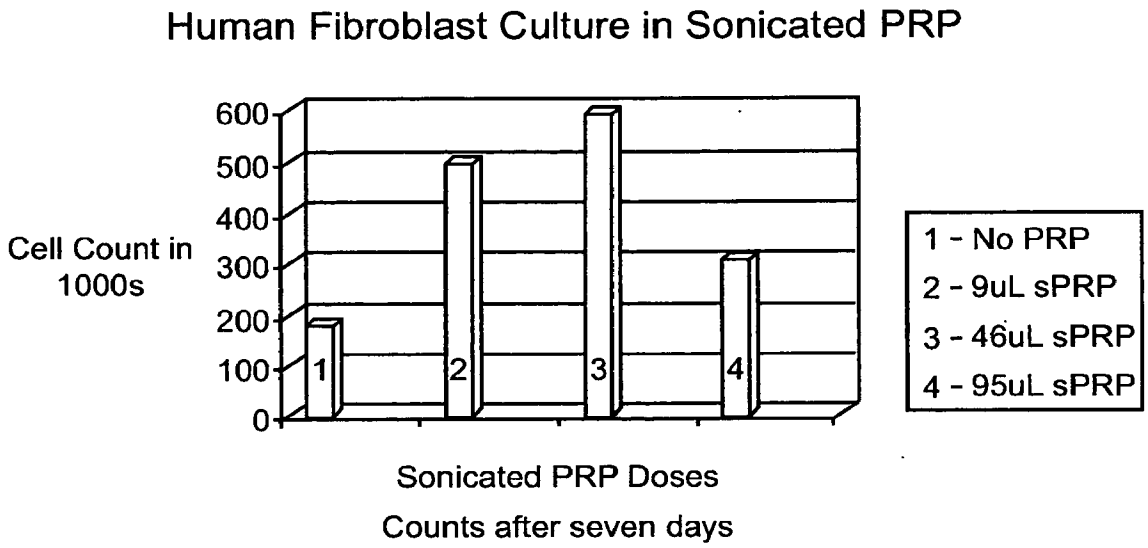
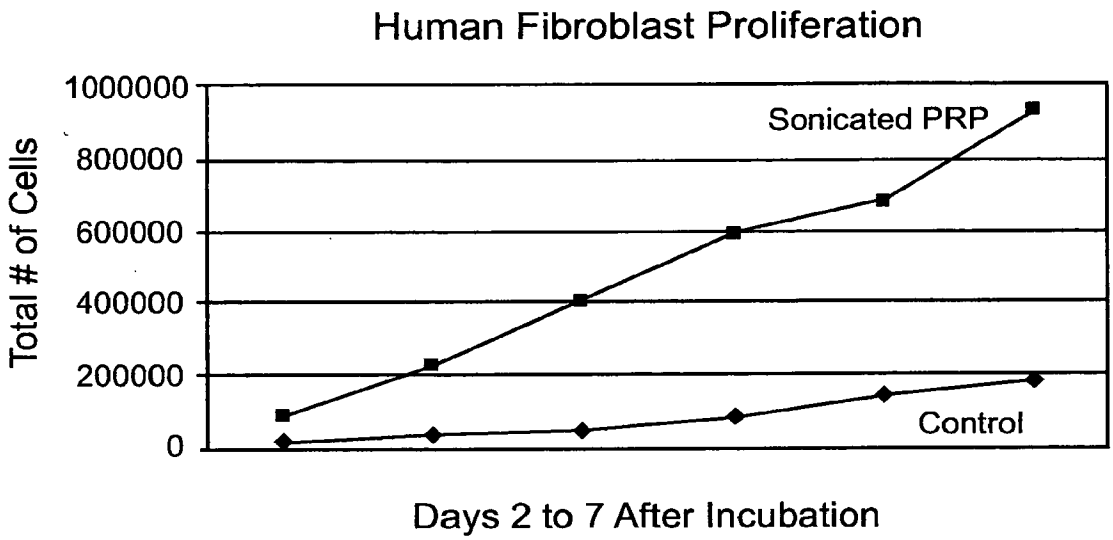

Human Fibroblast Culture in Sonicated PRP
Cell Count in 1000s
600
500
400
300
200
100
0
1
2
3
4
1 - No PRP
2 - 9uL sPRP
3 - 46uL sPRP
4 - 95uL sPRP
Sonicated PRP Doses
Counts after seven days

FIG. 3


Human Fibroblast Proliferation
1000000
800000
600000
400000
200000
0
Total # of Cells
Sonicated PRP
Control
Days 2 to 7 After Incubation

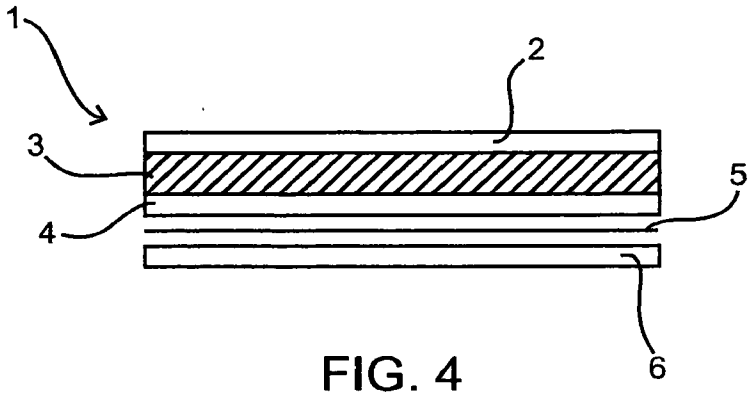


FIG. 4

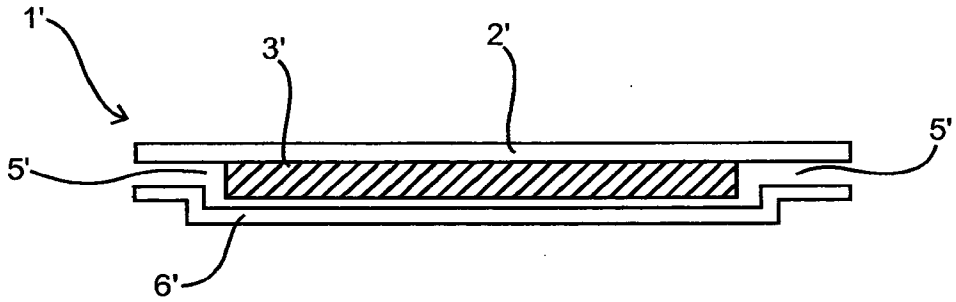


FIG. 5

FIG. 6

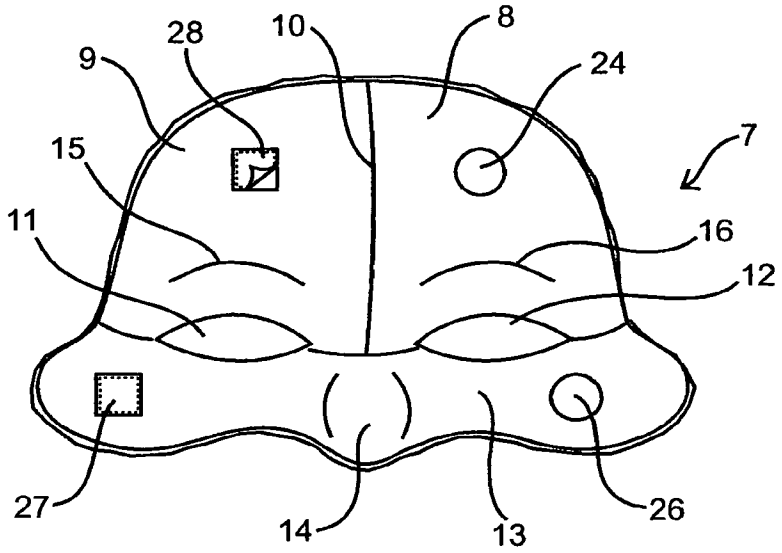
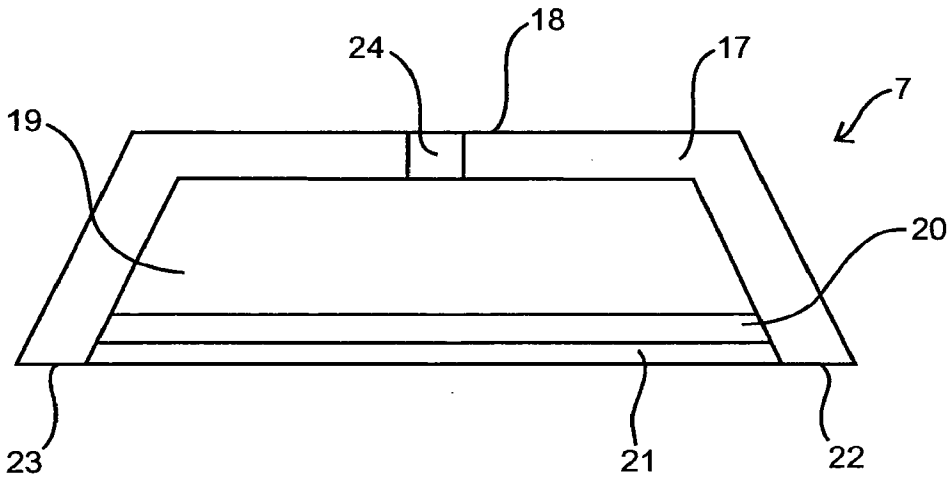


FIG. 7



APPLICATION INFORMATION

Application Type::	Provisional
Title::	PATIENT INDIVIDUALIZED TRANSDERMAL PATCH
Attorney Docket Number::	AMBI-006PRV
Request for Non-Publication?::	No
Assignee for Publication::	AM Biosolutions
Total Drawing Sheets::	5
Small Entity?::	Small
License US Govt. Agency::	No
Contract or Grant Numbers::	N/A
Sequence Submission?::	No
Computer Readable Form (CRF)?::	No

INVENTOR INFORMATION

Inventor One Given Name::	Allan
Family Name::	Mishra
Postal Address Line One::	1312 Orange Avenue
Postal Address Line Two::	
City::	Menlo Park
State or Province::	California
Postal or Zip Code::	94025
Citizenship Country::	USA

Inventor Two Given Name::	
Family Name::	
Postal Address Line One::	
Postal Address Line Two:	
City::	
State or Province::	
Postal or Zip Code::	
Citizenship Country::	

Inventor Three Given Name::	
Family Name::	
Postal Address Line One::	
Postal Address Line Two::	
City::	
State or Province::	
Postal or Zip Code::	
Citizenship Country::	

CORRESPONDENCE INFORMATION

Correspondence Customer Number::	24353
Telephone One::	(650) 327-3400
Telephone Two::	(650) 833-7735
Fax::	(650) 327-3231
Electronic Mail::	bozicevic@bozpat.com

REPRESENTATIVE INFORMATION

Representative Customer Number::	24353

CONTINUITY INFORMATION

This application is a::
> Application One::
Filing Date::

This application is a::
> Application Two::
Filing Date::

which is a::
>> Application Three::
Filing Date::

which is a::
>>> Application Four::
Filing Date::

PRIOR FOREIGN APPLICATIONS

Foreign Application One::
Filing Date::
Country::
Priority Claimed::